

## SEROTONIN IN PORIFERA? EVIDENCE FROM DEVELOPING *TEDANIA IGNIS*, THE CARIBBEAN FIRE SPONGE (DEMOSPONGIAE)

SIMON WEYRER, KLAUS RÜTZLER AND REINHARD RIEGER

Weyrer, S., Rützler, K. & Rieger, R. 1999 06 30: Serotonin in Porifera ? Evidence from developing *Tedania ignis*, the Caribbean fire sponge (Demospongiae). *Memoirs of the Queensland Museum* 44: 659–665. Brisbane. ISSN 0079–8835.

Histochemical study of larvae and freshly settled juveniles of the Caribbean fire sponge *Tedania ignis* (Tedaniidae, Poecilosclerida) reveals evidence of serotonin-like immuno-reactivity, a possible indication for the presence of precursors of nerve cells in this species. Already in the earliest stages of its life, *T. ignis* is made up of two discernable cell types: monociliated cells arranged in quasi-epithelial fashion and covering the larva and the developing settled organism, and mesohyal cells (archoocytes). In the adult sponge, several mesohyal cell types can be distinguished which form a complex connective tissue. Serotonin-like immuno-reactivity demonstrated by us occurs only in two cell types: in some archoocytes of the parenchymella larvae, and in similar archoocytes and in a second, bipolar cell type of the settled, juvenile sponge. The discovery of a neuroactive substance in cells of developing sponges before and after metamorphosis provides new insights into the origin and evolution of nerve and muscle cells in the Eumetazoa. □ *Porifera, histochemistry, serotonin, Tedania ignis, larval development, evolution.*

Simon Weyrer & Reinhard Rieger, Institute of Zoology and Limnology, University of Innsbruck, Technikerstrasse 25, A-6020 Innsbruck, Austria; Klaus Rützler (email: ruetzler@nmnh.si.edu), Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington D.C. 20560, USA; 18 January 1999.

Although sponges, one of the oldest metazoan groups, possess the greatest diversity of biologically active compounds of any marine phylum, the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) has been reported only once, in myocyte-like cells of *Sycon ciliatum* (Sycettidae, Calcarea) (Lenz, 1966). Serotonin appeared early in the evolution of eucaryotes. For example, it is used in chemical signal chains in Protista where, in a species of the ciliate *Blepharisma*, a serotonin-like substance is known to function as a mating pheromone (Haldane, 1954; Miyake, 1984). It has also been shown that a number of lower organisms use serotonin as an internal messenger in their neurotransmitter-receptor systems (Carr et al., 1989) and that some of these characteristics of molecular structure that arose in unicellular organisms may have been inherited and modified by metazoans (Mackie, 1990; Van Houten, 1990).

It seems obvious that nerve cells developed gradually over a long period of time but the sequences of changes that must have occurred are difficult to establish. Being a primitive outgroup of the Eumetazoa, Porifera do not have neurons or myocytes that are present in organisms of higher levels of organisation. A common phylogenetic hypothesis such as the Planula or

Phagocytella hypothesis (see Hyman, 1951; Ivanov, 1988; Rieger et al., 1991; Ax, 1995) encouraged the authors to search for precursors of nerve and muscle cells in sponge larvae in early developmental stages rather than in adults, a neglected area of research so far (Harrison & De Vos, 1991; Woollacott & Pinto, 1995). Such precursors of nerve cells and myocytes in sponges could represent the first stage in the evolution of integrative systems (e.g. Pavans de Ceccaty, 1974a, 1989; Mackie, 1990).

### MATERIALS AND METHODS

Larvae of *Tedania ignis* (Durchassaing & Michelotti) (Tedaniidae, Poecilosclerida, Demospongiae) were sampled in the laboratory seawater system of the Smithsonian Coral Reef Field Station at Carrie Bow Cay, Belize, in March 1994 and November 1995. Larval release was induced in adult specimens collected in the nearby mangrove of Twin Cays (Rützler & Feller, 1996) and maintained in aerated seawater by exposing them to natural sunlight following a 12–24hr period of dark adaptation (Woollacott, 1993). The larvae were kept in seawater-rinsed glass dishes (10cm diameter) and fixed immediately after release and 80–100hrs after attachment to the substrate. To provide a

substrate suitable for fixation and removal for subsequent processing, the bottom of these dishes was coated with polymerised epoxy resin (Spurr).

Specimens were fixed in 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS; 0.1M, pH 7.4) for 6–8hrs, rinsed in PBS, and treated with Triton X-100 (0.2%, 1hr) to permeabilise membranes. After labelling with the primary antibody (rabbit anti-serotonin, IMMUNOTECH 0601; 2.5%) overnight at 4°C, fluorescence-labelling was done for 1hr with a tetra-rhodamine-isothiocyanate-(TRITC)-conjugated secondary antibody (swine anti-rabbit; DAKO, 1%) for 1hr. Specimens were then rinsed in PBS, whole-mounted (Gelmount) on slides, and examined under a REICHERT Polyvar epifluorescence microscope. Incubation in bovine-serum albumin-Triton (BSA-T) without primary antibody was used as the control for nonspecific binding of the secondary antibody. Three larvae and three settled juvenile sponges were sectioned (epoxy-embedded, 1µm thick, stained with toluidin blue) and investigated in detail. The immuno-staining of both larvae and freshly settled sponges was carried out by the labelled streptavidin-biotin method (LSAB kit; DAKO). Histochemical staining of peroxidase with amino-ethyl-carbazole (AEC, substrate buffer) was used to enhance visibility of the labelled cells.

## RESULTS

*Tedania ignis* has a parenchymella larva composed of two types of cells (Bergquist et al., 1979). Peripherally, flagellated epithelium-like cells cover the organism. This "epithelium" is monociliated and 10–25µm high. The free-swimming larva exhibits coordinated ciliary action. A distinct basal lamina and typical eumetazoan apical junctional complexes are apparently lacking (but see below). Interior, apparently motile mesenchymal cells (mesohyl cells) are arranged beneath the epithelium-like sheath (Woollacott, 1990, 1993; Amano & Hori, 1994) (Fig. 1A). The live larvae are ovoid and have a size of 700–900µm long, 500–600µm wide, but the necessary Triton X-100 treatment weakens the cell membranes and larvae usually shrink and collapse (Fig. 1A, B). In the juvenile, settled sponge too—as in the adult—the exopinacoderm which covers the ectosome acts as the protective layer. Inside the sponge, choanocyte chambers connected by canals lined with endopinacocytes

lie embedded among mesohyl cells (Fig. 2A). Using a whole-mount fluorescence technique, we found serotonin-like immuno-reactivity in special mesohyl cells of both larval and juvenile *T. ignis*. Spherical serotonergic cells appear to be randomly distributed and occur alone or in clusters (Figs 1B, 2B). In one larva, for example, 6 clusters of such serotonin positive cells were found, each composed of 2–4 single spherical cells with a diameter of 4–6µm. In some clusters as well as in several single cells the nuclei are clearly visible and appear as non-fluorescent regions (Fig. 1B).

In the juvenile, settled sponge, a few bipolar cells were found in addition to the spherical cells that superficially resemble bipolar neurons or 'myocyte-like' cells (actinocytes) reported by Bagby (1966) (Fig. 2B, C). These bipolar cells have a maximum length of 20–50µm. Both types of serotonin-positive cells (spherical and bipolar) appear to be located in the mesohyl as spicules can be seen on top of the labelled cells (Fig. 2C).

No information is yet available on whether interactions between these morphological types of serotonin-positive cells occur, nor do we know whether the bipolar cells differentiate from the spherical type. If these serotonergic cells are part of an integrative system, both cellular communication at a distance (spherical cells) or cell–cell contacts (bipolar cells) could be expected.

## DISCUSSION

Our study is the first to report serotonergic cells in Demospongiae, a spherical type in both larva and post-metamorphosed sponge, and a second bipolar cell type that is exclusive to the post-larval developing organism. Up to now, serotonin was only demonstrated histochemically in myocyte-like cells of *Calcarea* (see below). Among the most primitive Eumetazoa, serotonin is well known to act as a neurotransmitter (e.g. in Anthozoa, Umbriaco et al., 1990). Actually, 5-HT has a wide range of functions in invertebrates, such as control of regeneration processes in *Planaria* (Kimmel & Carlyon, 1990) and of beat of cilia in echinoderm embryo (Mogami et al., 1992), and as inhibitors and activators of muscle of molluscs (Welsh, 1953; Twarog, 1988). As in Porifera, members of the phylum Placozoa do not differentiate nerve or muscle cells and are therefore counted among the most primitive eumetazoans (Grell, 1974; Ax, 1989; Grell & Ruthmann, 1991). Schuchert (1993) demonstrated in *Trichoplax adherens*

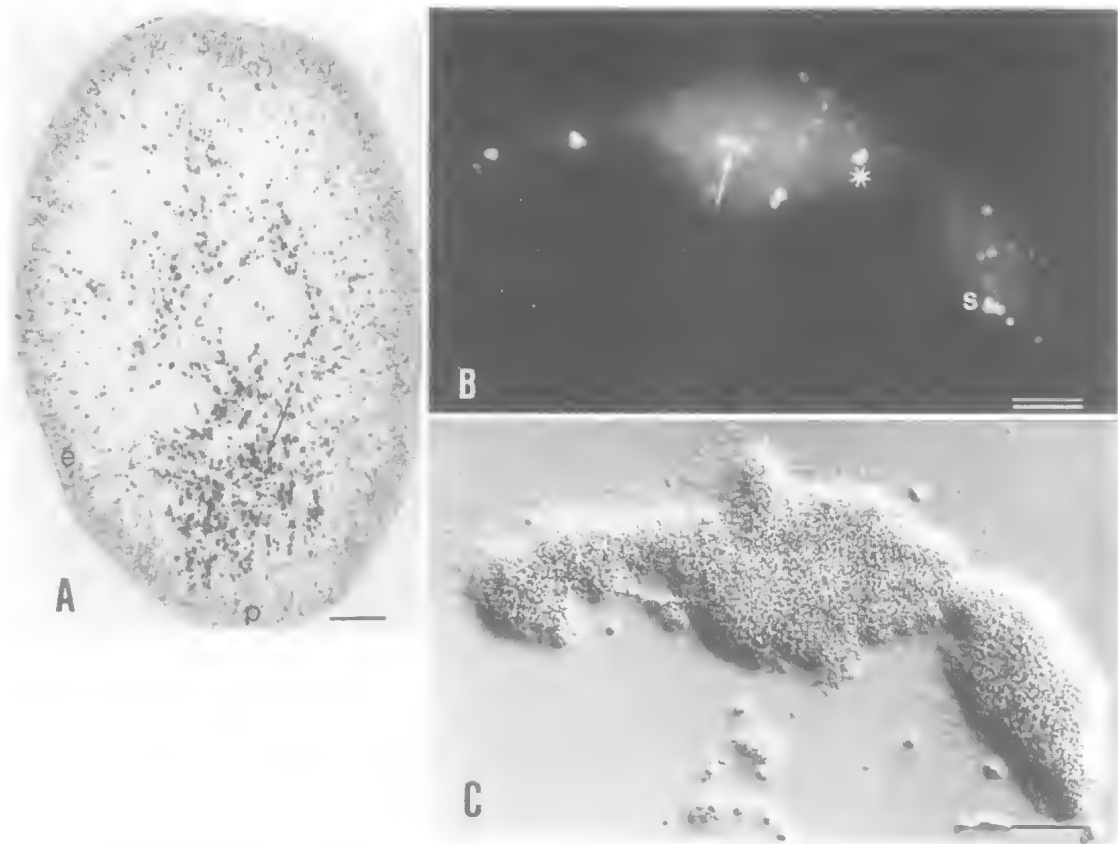


FIG. 1. *Tedania ignis*, histology of larva. A, Longitudinal section of an entire larva stained by toluidin blue showing epithelial-like cell layer (e) and dark-staining cells (archaeocytes, arrow) in clusters near the posterior pole (p) (scale bar=100 $\mu$ m). B, Serotonin-positive cells (s) are randomly distributed in the larval body; at least 6 clusters of 2-4 labelled cells are evident (one marked, asterisk). The nucleus in some of the cells is visible as a non-fluorescent region (arrow). As control for non specific binding of the secondary antibody specimen were incubated in BSA-T without primary antibodies. (Scale bar=100 $\mu$ m.) C, Nomarsky contrast view of the same specimen as in Fig. 1B. The collapsed and shrunk appearance is due to a necessary Triton X-100 treatment that weakens the cell membranes. (Scale bar=100 $\mu$ m.)

(Placozoa) bottle-shaped cells (2.7–4 $\mu$ m) that stain specifically with the neuropeptide RF-amide. The author speculated about a possible sensory function of the bottle-shaped cells because the RF-amide positive cells were localised at the margin of the disc-like body of *T. adhaerens* and the neuropeptide RF-amide is regarded as functionally conservative in lower invertebrates.

Much effort has been made toward identifying attachment complexes between adjacent cells of the pinacoderm in adult sponges, as this layer controls the sponge's internal milieu which differs from the surrounding environment (Ledger, 1975; for review see Harrison & De Vos, 1991). This altered chemical composition in the

tissue fluid of the sponge is regarded as a basic precondition for the evolution of nervous systems. Bandshaped, (epithelial-type) apical cell junctional complexes seem to be present in adult Porifera (e.g. apposed membrane junctions in Bagby, 1970; simple junctions in Ledger, 1975; parallel membrane junctions in Green & Bergquist, 1982; fig. 6 in Garonne & Lethias, 1990) in ultrastructural investigations of *Tedania ignis*, comparable structures seem to be evident (authors, unpublished). However, unequivocal clarification of the organisation of apical junctional complexes is still lacking in the Porifera. It has been stated repeatedly that permanent junctional complexes in epithelially organised cells, if present, are different in

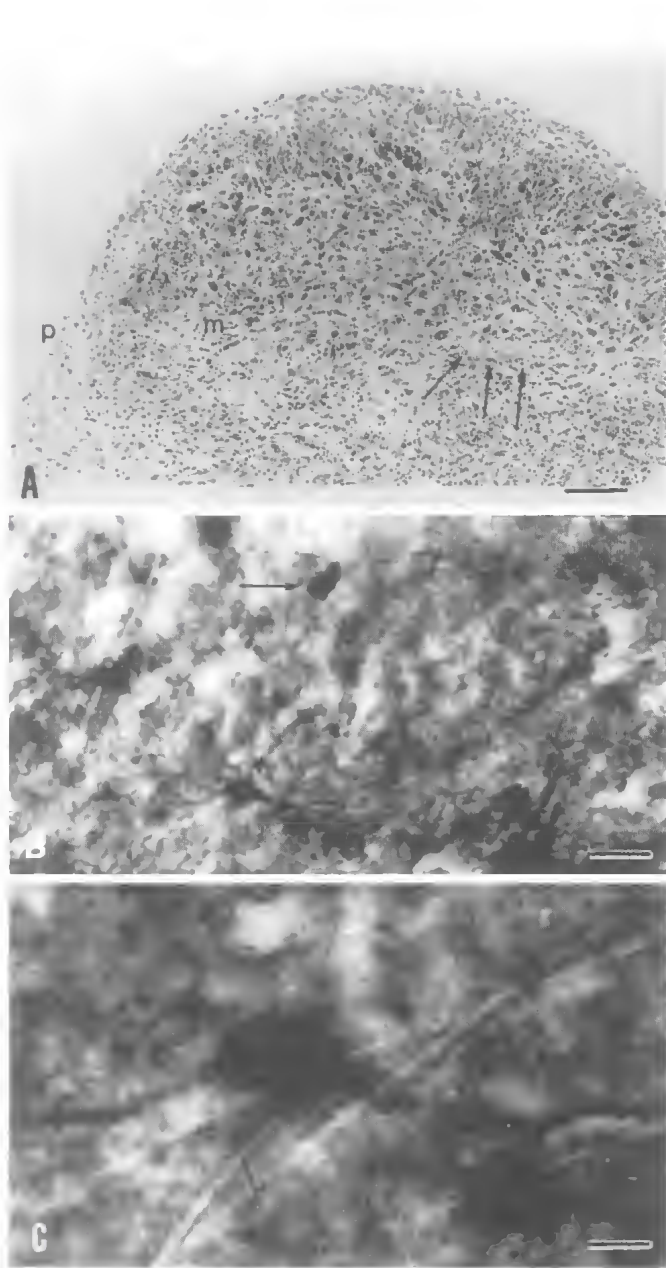


FIG. 2. *Tedania ignis*, histology of freshly metamorphosed juvenile sponge. A, Overview of a cross section stained by toluidin blue. The cells have begun to differentiate into pinacocytes (p) and multiple subtypes of mesohyl cells (m). A water canal in process of forming is indicated (arrow). (Scale bar=150 $\mu$ m.) B, Detail of the ectosome. Several spherical (arrow) and one bipolar (asterisk) serotonin-positive cells are visible. To enhance visibility of the labelled cells a histochemical peroxidase staining was used. (Scale bar=15 $\mu$ m.) C, Bipolar serotonergic cell (same specimen as in Fig 2A). Spicules (arrow) can be seen above the labelled cell. (Scale bar=4 $\mu$ m.)

construction from those of Metazoa (e.g. Green, 1978; Green & Bergquist, 1979). One reason for this difference is seen in the high motility and frequent rearrangement of sponge cells (e.g. Müller, 1982). However, it seems likely that development of permanent communicating structures goes hand in hand with less mobility and a highly differentiated state of cells. For example, Lethias et al. (1983) are of the opinion that additional freeze-fracture- and TEM-investigations of sponges might reveal membrane specialisations and connections to cytoskeletal components. On the other hand, several investigators of freshwater and marine sponges could not identify such junctional complexes (e.g. Lethias et al., 1983; Weissenfels, 1990; Woollacott, 1990).

While there is yet no evidence for chemical synapses in sponges, several reports discuss gap junction-like structures in the Porifera that may function as electrical synapses. For example, Green & Bergquist (1979) interpreted structures observed by SEM as temporary intercellular communication canals. Also, Revel (1988) and Garrone & Lethias (1990) describe various particle fields in freeze-fracture replicas, some of which superficially resemble gap junctions or rhomboid panicle fields of Eumetazoa but cannot unequivocally be claimed as typical gap junctions.

Contractile cells in the mesohyl of sponges—actinocytes (see Boury-Esnault & Rützler, 1997), but often called myocytes or myocyte-like cells—are arranged in networks (Bagby, 1966; Prosser, 1967; Bergquist, 1978; Burlando et al., 1984; Wachtmann et al., 1990; Harrison & DeVos, 1991) and have been seen as an early stage in the evolution of nerve and muscle cells (see literature in Lentz, 1966; Pavans de Ceccatty, 1974a; Mackie, 1990). Two types of microfilaments, that is, thin (5-7nm) and thick (15-25nm) filaments can be observed in the cytoplasm. In *Tedania ignis* and *Hippospongia communis*, only thick filaments have been reported (Harrison

& De Vos, 1991). The ability to contract or condense in response to endogenous events or external stimuli is a common feature in Calcarea and Demospongiae. It results in a decrease in body size and concurrent increase in number of cell contacts (review in Simpson, 1984; Weissenfels, 1989). One can speculate whether the increased number of cell contacts is only the result of a reduced body volume or possibly serves the intensification of 'signal transduction' in the network of actinocytes. Frequent cell-cell contacts between myocyte-like cells were also observed in *H. communis* (Pavans de Ceccatty et al., 1970). According to Pavans de Ceccatty (1974a), the microfilament-containing pinacocytes play an important role in this process, both for cell contraction and cell communication. Owing to the dynamic, 'loose' organisation of cellular features (Pavans de Ceccatty, 1974b; Bond, 1992) there are no nervous cells evident in sponges, but one can expect temporary, fixed pathways through connected mesohyl cells at the points of stable intercellular connections. Lentz (1966) reported acetylcholinesterase, monoamine oxidase, epinephrine, norepinephrine, and 5HT (serotonin) in 'myocyte-like' cells of *Sycon ciliatum*. These observations along with the association of cholinesterase and myofilaments in myocyte-like cells and the report of actin filaments in pinacocytes (Pavans de Ceccatty, 1989) may signify myoid and neuroid elements from a common integrative system that is coordinating 'tissue' contractions in sponges although electrophysiological evidence of a conducting mechanism is still lacking (Lawn, 1982).

In conclusion, we believe our findings of serotonergic cells in the Parazoa suggest an evolutionary specialisation of serotonin, separate from its function in Protists. We interpret our observations as supporting the recently emphasised sister-group relationship with the Eumetazoa (Morris, 1993; Müller, 1995; Ax, 1995) by exhibiting the very first steps in the evolutionary development of the integrative system of the Metazoa. Further research involving additional species and immunocytochemical, electrophysiological, and other approaches is clearly needed.

#### ACKNOWLEDGMENTS

We are indebted to G. Rieger, D. Reiter, P. Ladurner (University of Innsbruck), and W.E.G. Müller (University of Mainz, Germany) for

valuable discussions and for critical reading of the manuscript. We thank K.P. Smith (Dept. of Invertebrate Zoology, National Museum of Natural History, Washington, D.C.) and M.C. Diaz (University of California, Santa Cruz) for help with fieldwork in Belize. The staff of the Department of Ultrastructure and Evolutionary Biology of the University of Innsbruck are thanked for the use of facilities and technical advice (W. Salvenmoser, in particular). This paper was presented at the 5th International Sponge Symposium in Brisbane, 1998, and we wish to acknowledge British Airways for the donation of guest tickets to the Smithsonian Institution which allowed K. Rützler and K.P. Smith to attend. Research in Belize, Innsbruck, and Washington was supported by grants from Fond Wissenschaftlicher Forschung (Austria) and funds from the Caribbean Coral Reef Ecosystems Program (CCRE), National Museum of Natural History, Smithsonian Institution (contribution no. 484).

#### LITERATURE CITED

- AMANO, S. & HORI, I. 1994. Metamorphosis of a demosponge. I. Cells and structure of swimming larval. *Invertebrate Reproduction and Development* 25: 193-204.
- AX, P. 1989. Basic phylogenetic systematization of the Metazoa. Pp. 229-245. In Fernholm, B., Bremer, K. & Joernvall, H. (eds) *The Hierarchy of Life*. (Elsevier: Amsterdam.)
1995. *Das System der Metazoa I*. (Gustav Fischer Verlag: Stuttgart, Jena, New York.)
- BAGBY, R.M. 1966. The fine structure of myocytes in the sponges *Microciona prolifera* (Ellis and Solander) and *Tedania ignis* (Durchassaing and Michelotti). *Journal of Morphology* 118:167-182.
1970. The fine structure of pinacocytes in the marine sponge *Microciona prolifera* (Ellis and Solander). *Zeitschrift für Zellforschung und mikroskopische Anatomie* 105: 579-594.
- BERGQUIST, P.R. 1978. *Sponges*. (Hutchinson: London.)
- BERGQUIST, P.R., SINCLAIR, M.E., GREEN, C.R. & SILYN-ROBERTS, H. 1979. Comparative morphology and behaviour of larvae of Demospongiae. Pp. 103-111. In Lévi, C. & Boury-Esnault, N. (eds) *Biologie des Spongiaires, Colloques Internationaux de CNRS Number 291*. (Centre National de la Recherche Scientifique: Paris.)
- BOURY-ESNAULT, N. & RÜTZLER, K. (eds) 1997. *Thesaurus of Sponge Morphology*. Smithsonian Contributions to Zoology 596: 1-55.
- BOND, C. 1992. Continuous cell movements rearrange anatomical structures in intact sponges. *Journal of Experimental Zoology* 263: 284-302.

- BURLANDO, B.E., GAINO, E. & MARCHISIO, P.C. 1984. Actin and tubulin in dissociated sponge cells. Evidence for peculiar actin containing microextensions. *European Journal of Cell Biology* 35: 317-321.
- CARR, W.E.S., GLEESON, R.A. & TRAPIDO-ROSENTHAL, H.G. 1989. Chemosensory systems in lower organisms: Correlations with internal receptor systems for neurotransmitters and hormones. *Advances in Comparative Environmental Physiology* 5: 25-52.
- GARRONE, R. & LETHIAS, C. 1990. Freeze-fracture study of sponge cells. Pp. 121-128. In Rützler, K. (ed.) *New Perspectives in Sponge Biology*. (Smithsonian Institution Press; Washington, D.C.)
- GREEN, C.R. 1978. Variations in septate junction structure in the invertebrates. Pp. 338-339. In Sturgess, J.M. (ed.) *Electron Microscopy II*. (Microscopical Society of Canada; Toronto.)
- GREEN, C.R. & BERGQUIST, P.R. 1979. Cell membrane specializations in the Porifera. Pp. 153-158. In Lévi, C. & Boury-Esnault, N. (eds) *Biologie des Spongiaires, Colloques Internationaux du CNRS Number 291*. (Centre National de la Recherche Scientifique; Paris.)
1982. Phylogenetic relationships within the Invertebrata in relation to the structure of septate junctions and the development of "occluding" junctional types. *Journal of Cell Science* 53: 279-305.
- GRELL, K.G. 1974. Vom Einzeller zum Vielzeller. Hundert Jahre Gastrea-Theorie. *Biologie in unserer Zeit* 4: 65-71.
- GRELL, K.G. & RÜTHMANN, A. 1991. A new culture method for *Trichoplax adherens* F. E. Schulze. *Cytobiology* 4: 216-240.
- HALDANE, J.B. 1954. La signalisation animale. *Année Biologique* 58: 89-98.
- HARRISON, F.W. & DE VOS, L. 1991. Porifera. Pp. 29-89. In Harrison, F.W. & Westfall, J.A. (eds) *Microscopic Anatomy of Invertebrates*, 2: Placozoa, Porifera, Cnidaria, and Ctenophora. (Wiley-Liss: New York.)
- HYMAN, L.H. 1951. *The Invertebrates*. II. Platyhelminthes and Rhynchocoela. The acoelomate Bilateria. (McGraw Hill: New York.)
- IVANOV, A.V. 1988. On the early evolution of the Bilateria. *Fortschritte der Zoologie* 36: 349-352.
- KIMMEL, H.D. & CARLYON, W.D. 1990. Persistent effects of a serotonin depletor (p-chlorophenylalanine) in regenerated planaria (*Dugesia dorotocephala*). *Behavioral Neuroscience* 104: 127-34.
- LAWN, I.D. 1982. Porifera. Pp. 49-72. In Shelton, G.A.B. (ed.) *Electrical Conduction and Behaviour in "Simple" Invertebrates*. (Clarendon Press: Oxford.)
- LEDGER, P.W. 1975. Septate junctions in the calcareous sponge *Sycon ciliatum*. *Tissue and Cell* 7: 13-18.
- LENTZ, T.L. 1966. Histochemical localisation of neurohumors in a sponge. *Journal of Experimental Zoology* 162: 171-180.
- LETHIAS, C., GARRONE, R. & MAZZORANA, M. 1983. Fine structure of sponge cell membranes: Comparative study with freeze-fracture and conventional thin section methods. *Tissue and Cell* 15: 523-535.
- MACKIE, G.O. 1990. The elementary nervous system revisited. *American Zoologist* 30: 907-920.
- MIYAKE, A. 1984. Blepharismone: A conjugation-inducing tryptophan derivate in the ciliated *Blepharisma*. Pp. 807-813. In Schlossberger, H., G., Hocken, W., Linzen, B. & Steinhart, H. (eds) *Progress in Tryptophan and Serotonin Research*. (De Gruyter; Berlin.)
- MOGAMI, Y., WATANABE, K., OOSHIMA, C., KAWANO, A. & BABA, S.A. 1992. Regulation of ciliary movement in sea urchin embryos: Dopamine and 5-HT change the swimming behavior. *Comparative Biochemistry and Physiology* 101C: 251-254.
- MORRIS, P.J. 1993. The developmental role of the extracellular matrix suggests a monophyletic origin of the Kingdom Animalia. *Evolution* 47: 152-165.
- MÜLLER, W.E.G. 1982. Cell membranes in sponges. *International Review of Cytology* 77: 129-181.
1995. Molecular phylogeny of Metazoa (Animals): Monophyletic origin. *Naturwissenschaften* 82: 321-329.
- PAVANS DE CECCATTY, M. 1974a. The origin of the integrative system: A change in view derived from research on Coelenterates and Sponges. *Perspectives in Biology and Medicine* 17: 379-390.
- 1974b. Coordination in sponges: The foundation of integration. *American Zoologist* 14: 895-903.
1989. Les éponges, à l'aube des communications cellulaires. *Pour la Science* 142: 64-72.
- PAVANS DE CECCATTY, M., THINEY, Y. & GARRONE, R. 1970. Les bases ultrastructurales des communications intercellulaires dans les oscules des quelques éponges. Pp. 449-466. In Fry, W.G. (ed.) *The Biology of the Porifera*. (Academic Press: London.)
- PROSSER, C.L. 1967. Problems in the comparative physiology of non-striated muscles. Pp. 133-149. In Wiersma, C.A.G. (ed.) *Invertebrate Nervous Systems: Their Significance for Mammalian Neurophysiology*. (University of Chicago Press: Chicago.)
- REVEL, J.P. 1988. The oldest multicellular animal and its junctions. Pp. 135-149. In Hertzberg, E.L. (ed.) *Gap Junctions*. (A.R. Liss: New York.)
- RIEGER, R.M., HASZPRUNAR, G. & SCHUCHERT, P. 1991. On the origin of the Bilateria: Traditional views and new concepts. Pp. 107-113. In Simonetta, A.M. & Conway Morris, S. (eds.) *The Early Evolution of Metazoa and the Significance of Problematic Taxa*. (Clarendon Press: Oxford.)

- RÜTZLER, K. & FELLER, C. 1996. Caribbean mangrove swamps. *Scientific American* 274(3): 94-99.
- SCHUCHERT, P. 1993. *Trichoplax adherens* (Phylum Placozoa) has cells that react with antibodies against the neuropeptide RF-amide. *Acta Zoologica* 74: 115-117.
- SIMPSON, T.L. 1984. The cell biology of sponges. (Springer-Verlag: New York.)
- TWAROG, B.M. 1988. Serotonin: History of a discovery. *Comparative Biochemistry and Physiology* 91: 21-24.
- UMBRIACO, D., ANCTIL, M. & DESCARRIES, L. 1990. Serotonin-immunoreactive neurons in the cnidarian *Renilla koellikeri*. *Journal of Comparative Neurology* 291: 167-78.
- VAN HOUTEN, J. 1990. Chemoreception in unicellular eucaryotes. Pp 343-356. In Anderson, P.V.A. (ed.) *Evolution of the First Nervous Systems*. (Plenum: New York.)
- WACHTMANN, D., STOCKEM, W. & WEISSENFELS, N. 1990. Cytoskeletal organization and cell organelle transport in basal epithelial cells of the freshwater sponge *Spongilla lacustris*. *Cell and Tissue Research* 216: 145-154.
- WEISSENFELS, N. 1989. *Biologie und mikroskopische Anatomie der Süßwasserschwämme (Spongillidae)*. (Gustav Fischer Verlag: Stuttgart & New York.)
1990. Condensation rhythm of fresh-water sponges (Spongillidae, Porifera). *European Journal of Cell Biology* 53: 373-383.
- WELSH, J.H. 1953. Excitation of the heart of *Venus mercenaria*. *Archives of Experimental Pathology and Pharmacology* 219: 23-29.
- WOOLLACOTT, R.M. 1990. Structure and swimming behaviour of the larva of *Halichondria melanadocia* (Porifera: Demospongiae). *Journal of Morphology* 205: 135-145.
1993. Structure and swimming behaviour of the larva of *Haliclona tubifera* (Porifera: Demospongiae). *Journal of Morphology* 218: 301-321.
- WOOLLACOTT, R.M. & PINTO, R.L. 1995. Flagellar basal apparatus and its utility in phylogenetic analyses of the Porifera. *Journal of Morphology* 226: 247-265.